NOD/SCID mice and inosculate with murine vessels to participate in murine systemic blood flow. While CD34+CD45+ cells are enriched in hematopoietic colony forming cell activity, only CD34+CD45- cells give rise to ECFC in vitro. Recent studies indicate that ECFC can be derived from umbilical arterial and venous endothelial cells as well as endothelium from several other vascular sites. Thus, umbilical cord blood and blood vessels may be sources of cells that can be used for vascular regeneration.

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### DIFFERENTIATION OF UCB-DERIVED MULTI-LINEAGE PROGENITOR CELLS INTO RESPIRATORY EPITHELIAL CELLS

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Umbilical cord blood (UCB) recently has been examined for the presence of stem cells capable of differentiating into cell types representative of all three embryonic layers. The few groups reporting success have typically used hepatic differentiation for demonstration of endodermal potential. We report differentiation of human UCB-derived stem cells called multi-lineage progenitor cells, or MLPCs, into respiratory epithelial cells. Using a cell separation medium (PrepaCyte-MLPC; BioE, Inc.) and plastic adherence, MLPCs were isolated from UCB units (American Red Cross Cord Blood Program) and expanded. Cultures were grown in MSCGM (Cambrex, Inc.) prior to addition of Small Airway Growth Medium (SAGM; Cambrex, Inc.), a maintenance medium designed for airway epithelium. Following an 8 day culture, cells were characterized by light microscopy, transmission electron microscopy, immunofluorescence (IF), and PCR. Differentiated cells were characterized by an epithelioid morphology with lamellar bodies, the organelles responsible for secretion of surfactant. IF and PCR confirmed presence of protein product and mRNA of surfactant protein C, a protein highly specific for type II cells. UCB-derived MLPCs were isolated and expanded and then differentiated into respiratory epithelial cells using an off-the-shelf medium designed for maintenance of fully differentiated respiratory epithelial cells. To the best of our knowledge, this is the first time human non-embryonic stem cells have been differentiated into type II alveolar cells. Additional studies are ongoing to further evaluate the cells and the possibilities for therapeutic applications.

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### PROGRESS IN REGENERATIVE MEDICINE USING CORD BLOOD: MESENCHYMAL STEM CELL ISOLATION AND INDUCTION TO BONE AND CARTILAGE

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Although experimental and clinical trials using bone marrow derived mesenchymal stem cells demonstrated promising results, cells from other sources have also been explored. We report the isolation, expansion and differentiation of mesenchymal stem cells (MSCs) derived from human umbilical cord blood and compare the results to those of MSCs derived from human bone marrow, adipose tissue and chorionic villi of placenta. Though surface antigens of these various MSCs did not show significant differences, differentiation abilities were significantly different among these MSCs. Cord blood-derived MSCs (CB-MSCs) were isolated from 70% of cord blood units processed within 5 hours and at least 60 ml in volume. In each case these MSCs differentiated with high efficiency to chondrocyte lineages as well, or better, than those MSCs derived from bone marrow (BM-MSCs), but adipose tissue-derived MSCs (AD- MSCs) did not. In vivo transplantation of CB-MSCs with scaffolds, atero-collagen for chondrocyte or β-TCP for osteocytes into nude mice resulted in generation of chondrocyte-like tissue as well as bone. Co-culture of MSCs with lymphocytes stimulated with phytohemagglutinin and mixed lymphocyte culture suppressed proliferation of stimulated lymphocytes as well as BM-MSCs and AD-MSCs, indicating that CB-MSCs had immune-suppressive effect. Thus, the CB-MSCs can be a possible allogeneic cell source for regenerative medicine.

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### REDUCED RISK OF LEUKEMIA RELAPSE AFTER DOUBLE UCB TRANSPLANTATION

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Allogeneic hematopoietic cell transplantation (allo-HCT) is potentially curative therapy for patients with high-risk or relapsed acute leukemia. In the absence of a matched sibling donor, hematoipoietic progenitor cells can be obtained from either the bone marrow (BM), peripheral blood (PB) or umbilical cord blood (UCB) of volunteer donors. Compared to BM and PB, UCB has a number of benefits including the lack of collection risk to the donor, the absence of donor attrition and rapid availability. UCB also has less stringent requirements for HLA matching and in the majority of patients a suitably matched donor can be identified. However, the widespread applicability of UCB transplantation has been constrained by the limited volume of blood contained in within the umbilical cord. This small volume of blood translates into limited numbers of mononuclear cells and $CD34^+$ progenitor cells and the dose of these (per kg recipient body weight) is the single most import variable for UCB outcome. Thus, UCB has been reserved mainly for children. Recently, we have demonstrated that the addition of a second, partially HLA matched UCB unit can augment the cell dose; increasing both the rate and frequency of donor engraftment in patients who would otherwise have an inadequate cell dose with in a single UCB unit. Interestingly, the majority (>60%) of recipients of double UCB transplantation showed engraftment of a single UCB unit. By day 21 after transplant, the second unit was not detected and presumably eradicated due to immunological mechanisms. To date it is unknown whether the addition of a second, partially matched UCB unit has any impact on leukemia recurrence. We retrospectively reviewed the outcomes of patients with acute leukemia who received myeloablative conditioning chemotherapy followed by either single or double UCB transplantation. The goal was to provide an adequate cell dose/kg to patients. On this basis, patients received either single (n=72) or double UCB (n=54) unit transplantation. Recipients of two UCB units had a number of transplant related variables which differed from single unit recipients. Not surprisingly, double unit recipients were older and weighed more than single recipients. There were no significant differences between the single and double recipient groups with respect to disease (AML vs. ALL) and stage (CR1-2 vs. CR3-relapse). Most patients ($\sim 2/3^{rds}$ for each group) were in CR1-2. The total dose of infused MNCs or $CD34^+$ cells was not different between the two groups (for double UCB transplants this was the total of *both* UCB units). Two conditioning regimens were used during the course of this study. Single unit recipients received either TBI/Cytoxan/ATG followed by CSA/Methylprednisone (n=53) or Fludarabine/CTX/TBI followed by CSA/MMF (n=14). Recipients of double unit transplantation received only the fludarabine containing regimen. The kinetics of engraftment and incidence of TRM were not different between the two groups. Recipients of two UCB transplants had a significantly higher rate of aGVHD compared to recipients of single unit transplantation (RR=2.7, <0.01). The rate of cGVHD was not different between recipients of single and double UCB transplantation. The median follow up was 4.9 yrs (CI, 0.7-1.0) for single unit recipients and 2 yrs (CD, 0.7-5) for double unit recipients. Cox regression showed that the following variables were not associated with relapse: recipient age, gender, HLA disparity, CMV serostatus, cell dose, $CD34^+$ cell dose, $CD3^+$ cell dose, conditioning regimen, GVHD prophylaxis and aGVHD. However, recipients of two UCB units were *less* likely to experience relapse relative to single unit recipients (RR=0.4, p=0.06). Subgroup analysis showed that patients with early stage disease (CR1-2) benefited from double UCB transplant (RR=0.2, p=0.01). There was no difference in relapse for the advance patients (CD3-relapse) in